Yellow fever laboratory diagnostic testing in Africa

Interim guidance

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World Health Organization

1. Introduction

1.1 Background

The current yellow fever outbreaks in Angola and the Democratic Republic of the Congo have highlighted that timely laboratory confirmation of suspected yellow fever cases is an essential part of an effective response.

In 2010, yellow fever case definitions, including criteria for laboratory testing were established by a global expert consultation[1i]. This guidance builds on those yellow fever case definitions, clarifying which tests should be done in outbreak and non-outbreak situations.

Although laboratory testing is an essential part of making a yellow fever diagnosis, final confirmation should be done on a case-by-case basis including analysis of the clinical presentation, epidemiological context, and vaccination history.

1.2 Target audience

This document aims to provide guidance for laboratory staff providing diagnostic testing for yellow fever virus infection. It also provides information about laboratory diagnostics for clinical practitioners managing patients with suspected yellow fever and public health professionals engaged in yellow fever surveillance and control activities.

2. Laboratory diagnostic testing algorithms for countries at risk for yellow fever in Africa

2.1 Countries with outbreaks

During outbreaks, laboratory testing strategies should prioritize confirmation of new instances of local transmission and minimize the number of tests required to avoid overwhelming capacity. **Figure 1** provides a means of applying such a strategy.

The basic tests needed for laboratory confirmation of yellow fever during an outbreak are:

- **enzyme-linked immunosorbent assay (ELISA)** to measure yellow fever virus IgM. Other flaviruses- dengue virus, West Nile virus and Zika virus- may give a false positive yellow fever ELISA result so, an ELISA panel for other expected flaviviruses, (as determined by local epidemiology) should be performed as a differential diagnosis.
- **reverse transcription polymerase chain reaction (RT-PCR) for yellow fever virus.** This should be performed on samples collected within ten days of onset of symptoms.

All specimens should be transported to laboratories with appropriate patient information (e.g. age, sex, place of residence, onset of symptoms, vaccination history and travel history). Laboratory test results cannot be interpreted correctly without this information. Blood specimens should be tested as soon as possible, preferably within 24 hours of arrival at the laboratory. The time when blood is collected- that is the length of time after onset of symptoms- affects the interpretation of the results of both tests. Therefore it is important to remember that the time estimate is based on the history given by the patient and may not always be accurate.

Current laboratory tests cannot differentiate between yellow fever virus IgM stimulated by vaccination and that stimulated by infection with yellow fever wild-type virus. Therefore, the laboratory results in people who have received a yellow fever vaccine within 30 days must be interpreted with care (see Figure 2) and assessed on a case by case basis, considering the clinical presentation and epidemiological context along with the laboratory results.

If the national laboratory cannot perform appropriate serology testing for yellow fever virus IgM and/or RT-PCR, specimens should be shipped to a WHO regional reference laboratory (RRL) or the nearest recognized laboratory able to perform these tests (see Annex 1).

In districts where local transmission has not yet been confirmed, blood samples should be taken from all people with suspected cases of yellow fever. If the laboratory has reached maximum capacity, priority should be given to testing specimens from those areas where local transmission has not yet been confirmed. It is not essential to perform serology testing to differentiate between yellow fever and other flaviviruses on specimens from areas where local transmission has already been confirmed. However, all specimens should be properly stored for future analysis if needed.

A suspected case of yellow fever is laboratory-confirmed if the following criteria are met:

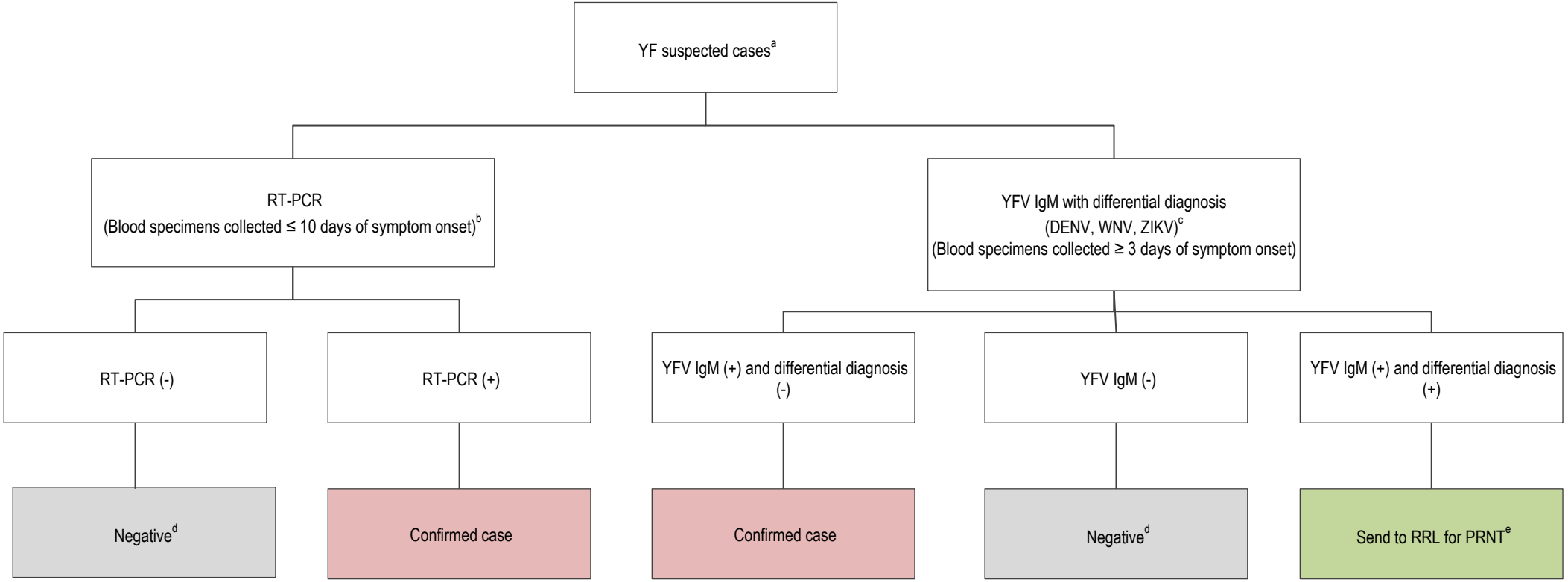
- presence of yellow fever virus RNA in blood from a person with no history of recent yellow fever vaccination[1]

or

- presence of yellow fever virus specific IgM antibody, absence of other relevant flaviviruses (dengue virus, West Nile virus, Zika virus) and no history of recent yellow fever vaccination.

[1] RT-PCR for yellow fever is currently validated for blood specimens only. Other specimens including saliva and urine may be validated for RT-PCR testing in the future.

Figure 1. Laboratory testing algorithm for suspected cases during yellow fever outbreaks: unvaccinated people



YF = yellow fever; YFV = yellow fever virus; DENV = dengue virus; WNV = West Nile virus; ZIKV = Zika virus; RRL = regional reference laboratory; PRNT = plaque reduction neutralization test

[a] Any person with acute onset of fever, with jaundice appearing within 14 days of onset of first symptoms[1]

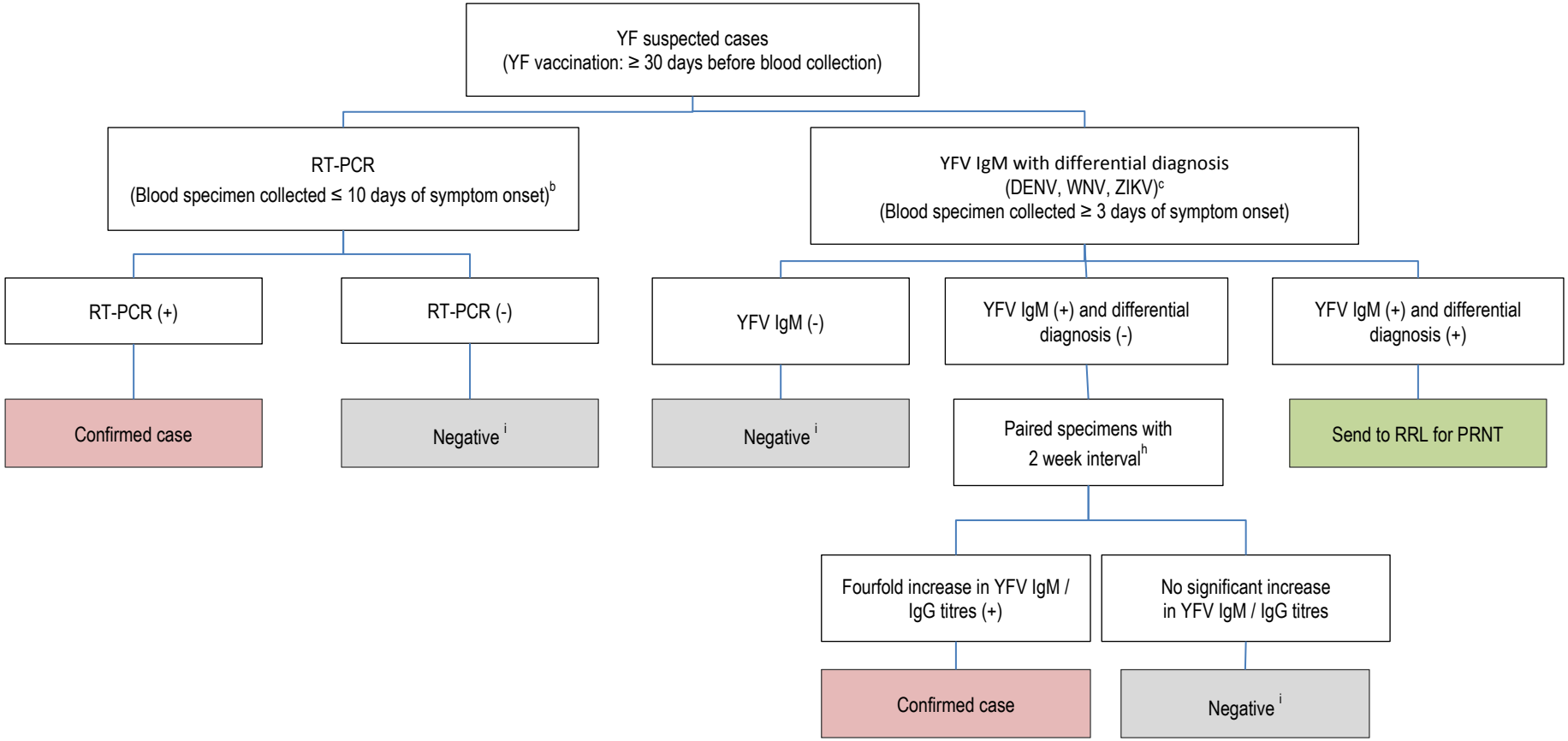
[b] If RT-PCR is conducted immediately after onset of symptom (< 3 days), negative cases should be retested 3 days after the onset of symptoms. In people with severe clinical symptom, RT-PCR may be positive for more than 10 days after the onset of symptoms. Urine testing is planned for the future but is not yet validated. When this is introduced it is important to know that when urine is tested by RT-PCR, the period of time after onset of symptoms during which the result may be positive, might exceed 10 days.

[c] Dengue virus, West Nile virus and Zika virus should be considered potential causative agents of symptoms and may test positive for YFV IgM. Depending on the local epidemiological situation, testing for other flaviviruses (ELISA) may need to be performed.

[d] When blood from people with suspected yellow fever is negative on both RT-PCR and YFV IgM testing, they are considered negative for YF. However, a negative result for only one of these tests does not rule out yellow fever infection.

[e] Plaque reduction neutralization test.

Figure 2. Laboratory testing algorithm for suspected cases during yellow fever outbreaks: people who have been vaccinated/people with unclear vaccination history[ii]



YF = yellow fever; YFV = yellow fever virus; DENV = dengue virus; WNV = West Nile virus; ZIKV = Zika virus; RRL = regional reference laboratory; PRNT = plaque reduction neutralization test

[b] If RT-PCR is conducted immediately after onset of symptom (< 3 days), negative cases should be retested 3 days after the onset of symptoms. In people with severe clinical symptom, RT-PCR may be positive for more than 10 days after the onset of symptoms. Urine testing is planned for the future but is not yet validated. When this is introduced it is important to know that when urine is tested by RT-PCR, the period of time after onset of symptoms during which the result may be positive, might exceed 10 days.

[c] Dengue virus, West Nile virus and Zika virus should be considered potential causative agents of symptoms and may test positive for YFV IgM. Depending on the local epidemiological situation, testing for other flaviviruses (ELISA) may need to be performed.

[h] This interval may be shortened, especially during outbreaks, as two days may be sufficient for a fourfold increase in YFV IgM titres if specific IgM antibody is released from the immune system[2,3,4].

[i] Yellow fever negative: (i) RT-PCR (-) and yellow fever virus IgM (-), or (ii) RT-PCR (-) and no significant increase in YFV IgM/IgG titres with two weeks interval.

[ii] This algorithm applies only to investigation of suspected cases in districts where local transmission has not yet been detected. In districts that have already confirmed local transmission it is not necessary to differentiate between yellow fever IgM caused by vaccination and that caused by wild YFV, therefore those districts should apply the algorithm in Figure 1.

2.2 At-risk countries with no current outbreak

In at-risk countries that do not have confirmed yellow fever outbreaks, laboratory testing should be used to detect a first (index) case.

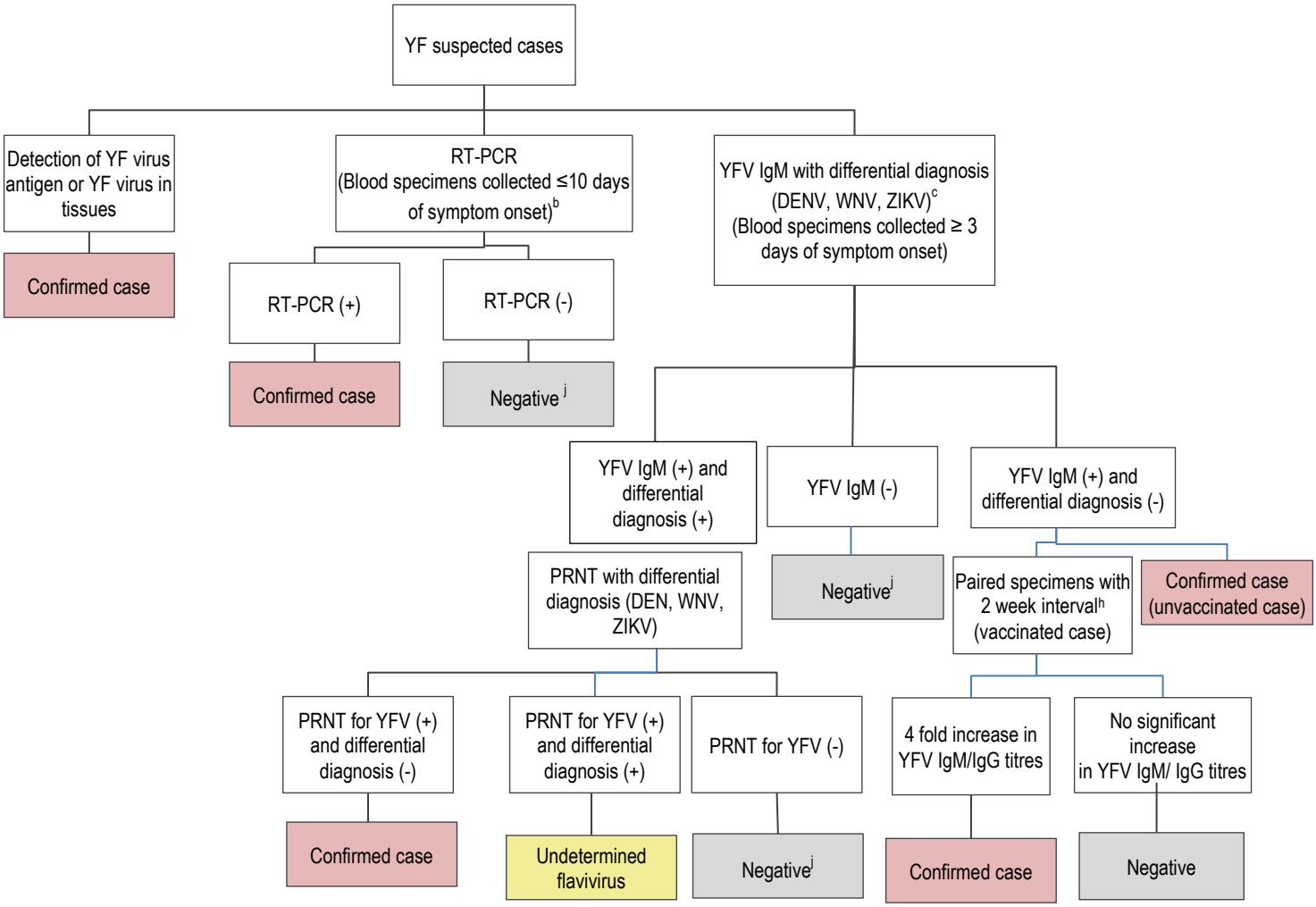
When blood samples are taken from people who have been recently vaccinated, or whose vaccination status is unknown, testing two samples- an initial acute and a later convalescent sample - can determine whether the presence of IgM is due to yellow fever virus infection. If there is a fourfold increase in the yellow fever virus IgM and/or IgG titres between the acute and convalescent serum specimens yellow fever infection can be confirmed.

In summary, a suspected new case of yellow fever can be laboratory confirmed by one of the following:

- presence of yellow fever virus RNA in blood taken from a person with no history of recent yellow fever vaccination; ***or***
- presence of yellow fever virus-specific IgM antibody and absence of other relevant flaviviruses (dengue virus, West Nile virus, Zika virus) without recent yellow fever vaccination history; ***or***
- a fourfold increase in yellow fever virus IgM and/or IgG titres between acute and convalescent blood specimens; ***or***
- presence of yellow fever neutralizing antibodies and absence of other flaviviruses (dengue virus, West Nile virus, Zika virus) in blood taken from a person with no history of yellow fever vaccination; ***or***
- detection of yellow fever antigen by immunoassay in tissues from a person with no history of recent yellow fever vaccination; ***or***
- isolation of yellow fever virus from blood or tissues from a person with no history of recent yellow fever vaccination.

(See Figure 3 below for more details)

Figure 3. Laboratory testing algorithm for suspected cases in non-outbreak settings



[b] If RT-PCR is conducted immediately after onset of symptom (< 3 days), negative cases should be retested 3 days after the onset of symptoms. In people with severe clinical symptom, RT-PCR may be positive for more than 10 days after the onset of symptoms. Urine testing is planned for the future but is not yet validated. When this is introduced it is important to know that when urine is tested by RT-PCR, the period of time after onset of symptoms during which the result may be positive, might exceed 10 days.

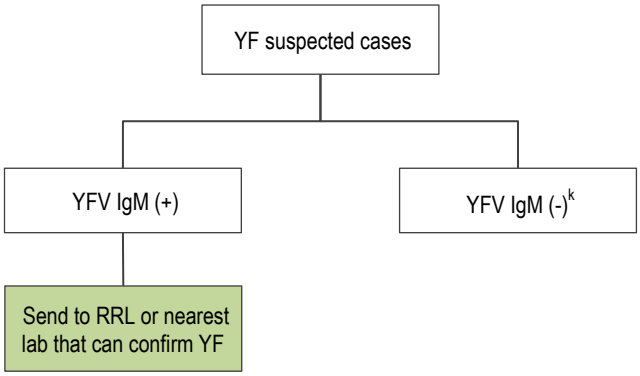
[c] Dengue virus, West Nile virus and Zika virus should be considered potential causative agents of symptoms and may test positive for YFV IgM. Depending on the local epidemiological situation, testing for other flaviviruses (ELISA) may need to be performed.

[h] This interval may be shortened, especially during outbreaks, as two days may be sufficient for a fourfold increase in YFV IgM titres if specific IgM antibody is released from the immune system[2,3,4].

[j] Yellow fever negative (i) PCR (-) and yellow fever virus IgM (-), or (ii) PCR (-) and PRNT for yellow fever (-)

If national laboratories cannot confirm yellow fever virus disease, specimens that have tested positive for yellow fever virus IgM should be shipped to a RRL as soon as possible (see Figure 4). When national laboratories introduce RT-PCR for yellow fever on a variety of samples (e.g. blood/serum, saliva and urine) and serology testing able to differentiate between flaviviruses, the algorithm will change.

Figure 4. Laboratory testing algorithm for suspected cases in non-outbreak settings where capacity to confirm yellow fever virus infection is limited



[k] If a blood specimen was collected within 7 days of symptom onset, a second specimen should be collected 7 days or more after symptom onset and retested.

3. Shipping specimens

The proper shipment of specimens to a regional reference laboratory with established laboratory networks (e.g. polio, measles, influenza, or the Emerging and Dangerous Pathogens Laboratory Network (EDPLN)[i]) requires advanced planning, appropriate packaging, labelling, documentation and communication between all parties involved. Specimens for molecular or serology testing should be kept at 4-8 ℃ and if they can be transported within one day to the diagnostic laboratory. If it is expected that transport will take more than one day, serum specimens should be frozen at -20 ℃. Improper handling of specimens will affect the quality of the diagnostic results.

See *Manual for the monitoring of yellow fever virus infection*[3] available at: http://apps.who.int/iris/bitstream/10665/68715/1/WHO_IVB_04.08.pdf

4. Indicators

The following indicators provide benchmarks for determining whether laboratory capacity is sufficient for supporting a yellow fever outbreak response.

Laboratory	Length of time taken (days)	Benchmark
National laboratory	Specimen arrived - specimen tested (yellow fever virus IgM with differential diagnosis, RT-PCR)	≤ 1day
	Specimen arrived – specimen shipped to RRL	≤ 3 days
Regional reference laboratories	Specimen shipped from national lab - results received from RRL (yellow fever virus IgM with differential diagnosis, RT-PCR)	≤ 5 days
	Specimen shipped from national lab - results received from RRL (PRNT)	≤ 10 days

5. Guidance development

5.1 Acknowledgements

This guidance was developed by an internal steering group made up of staff from WHO Geneva (Philippe Barboza, Mauricio Bellerferri, Pierre Formenty, Erika Garcia, Margaret Harris, Qiu Yi Khut, Miguel Norman Mulders, Dhamari Naidoo, Kyohei Nishino, Susan Norris, William Perea, and Sergio Yactayo); WHO Regional Office for Africa (Yahaya Ali Ahmed, Joseph Nsiari-Muzeyi Biey, Annick Ayélé Dosseh, Richard Ray Luce Jr and Jean-Bosco Ndihokubwayo); WHO Regional Office for the Americas (Jairo Andres Mendez Rico); WHO Regional Office for the Eastern Mediterranean (Humayun Asghar); WHO Regional Office for South-East Asia (Aparna Singh Shah); and WHO Regional Office for the Western Pacific (Franciscus Konings).

The external guideline development group was made up of the following experts who reviewed and revised the initial and the final draft: Maurice Demanou, Centre Pasteur Cameroon, Yaoundé, Cameroon; Barbara Johnson, Centers for Disease Control and Prevention, Atlanta, United States of America Koichi Morita, Institute of Tropical Medicine, Nagasaki University, Japan; Matthias Niedrig, Robert Koch-Institut, Berlin, Germany; Pedro Fernando da Costa Vasconcelos, Instituto Evandro Chagas, Belem, Brazil; and Herve Zeller, European Centre for Disease Prevention and Control, Stockholm, Sweden.

5.2 Guidance development methods

This guidance builds on the yellow fever case definition developed and published in 2010 (1). This guidance uses the case definition as agreed in 2010 but clarifies which tests should be done in outbreak and non-outbreak situations. An internal steering group (see acknowledgements above) made up of WHO staff in headquarters and regional offices developed the first draft. This was then circulated to an external review group made up of people with expertise in laboratory testing and

virology, from the Americas, Europe, and the Western Pacific region (see acknowledgements for full list), who were identified via WHO collaborating centre networks. The external review group reviewed the draft guidance via email and provided written reviews and comments which were incorporated into the revised document. This document was then reviewed by all participants for a second time and input received incorporated in the final document.

5.3 Declaration of interests

No competing interests were identified from the declarations of interests collected. No specific funds were used to develop this guidance.

5.4 Review date

These recommendations have been produced under emergency procedures and will remain valid until December 2016. The internal steering group who developed this guideline will be responsible for reviewing the contents at that time, and updating it as appropriate.

6. References

1. Weekly Epidemiological Record 2010. Geneva: World Health Organization; 2010. (http://www.who.int/wer/2010/wer8547.pdf accessed 26 May 2016)
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5. Manual for the monitoring of yellow fever virus infection. Geneva: World Health Organization; 2004. (http://apps.who.int/iris/bitstream/10665/68715/1/WHO_IVB_04.08.pdf, accessed 26 May 2016).

Annex 1. Laboratories for confirmation of yellow fever

Region	Laboratory (city, country)
Africa	**Institut Pasteur in Dakar (Dakar, Senegal)***
	International Centre for Medical Research in Franceville (Franceville, Gabon)
	Kenya Medical Research Institute (Nairobi, Kenya)
	National Institute for Communicable Diseases (Johannesburg, South Africa)
	Noguchi Memorial Institute for Medical Research (Accra, Ghana)
	Uganda Virus Research Institute (Entebbe, Uganda)
Americas	**Instituto Evandro Chagas (Belem, Brazil)***
	Instituto Nacional de Enfermedades Virales Humanas (Pergamino, Argentina)*
	Institut Pasteur in French Guiana (Cayenne, French Guiana)*
	Instituto Pedro Kouri (Habana, Cuba)*
	Centers for Disease Control and Prevention (Fort Collins, United States of America)*
	Centers for Disease Control and Prevention- Puerto Rico (San Juan, Puerto Rico-United States of America)*
	Instituto Nacional de Salud (Bogota, Colombia)
	Instituto Nacional de Salud (Lima, Peru)
Eastern Mediterranean	Central Public Health Laboratory (Cairo, Egypt)
	Central Public Health Laboratory (Khartoum, Sudan)
	Health Laboratory (Tehran, Iran)
	National Institute of Health (Islamabad, Pakistan)
	Public health laboratory (Manama, Bahrain)
	Rafiq Harairi Hospital (Beirut, Lebanon)
	Virology Lab (Rabat, Morocco)
Europe	**Robert Koch-Institut (Berlin, Germany)***
	Bernhard Nocht Institute for Tropical Medicine (Hamburg, Germany)
	The State Research Center of Virology and Biotechnology VECTOR (Novosibirsk, Russia)
	Institut Pasteur in Paris (Paris, France)
	Rare and Imported Pathogens Laboratory, Public Health England (London, the United Kingdom)
	Russian Research Anti-Plague Institute «Microbe» (Saratov, Russia)
South East Asia	National Institute of Virology (Pune, India)

***WHO regional reference laboratories for yellow fever**